J. Serb. Chem. Soc. 72 (8–9) 879–887 (2007) UDC 541.182.3+66.061:632.95:541.121:544.4
JSCS–3620 *Original scientific paper*

Headspace solid phase microextraction in the analysis of pesticide residues – kinetics and quantification prior to the attainment of partition equilibrium

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(Received 9 February 2006, revised 12 March 2007)

Abstract: A new theoretical approach to the headspace/solid phase microextraction (HS/SPME) process is proposed and tested by the analysis of pesticide residues of water samples. The new approach focuses on mass transfer at the sample/gas phase and gas phase/SPME polymer interfaces. The presented model provides a directly proportional relationship between the amount of analytes sorbed by the SPME fiber and their initial concentrations in the sample. Also, the expression indicates that quantification is possible before partition equilibrium is attained. Experimental data for pesticides belonging to various classes of organic compounds were successfully interpreted by the developed model. Additionally, a linear dependence of the amount of pesticide sorbed on the initial analyte concentration in aqueous solution was obtained for a sampling time shorter than that required to reach sorption equilibrium.

Keywords: HS/SPME, theoretical model, pesticide residues.

INTRODUCTION

Solid phase microextraction (SPME) is a solvent-free and equilibrium sample preparation technique in which a fused silica fiber coated with a thin polymer film is introduced into a sample or the headspace above the sample. After partitioning between the polymer layer and the sample matrix, organic analytes are selectively extracted by the active film. Developed by Pawliszyn and coworkers,[1,2] it has wide applications in the analysis of different types of organic residue samples of various origin. Using the headspace mode of SPME, complex matrix effects are reduced and the fiber lifetime is prolonged.

Hitherto, several theoretical models have been proposed to explain the SPME process. Pawliszyn and coworkers proposed models based on diffusion processes in both the direct and headspace modes.[3,4] The analytical solution was obtained only for perfectly agitated samples with an infinite volume where only the diffu-

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doi: 10.2298/JSC0709879D

sion inside the SPME fiber was considered.[3] In the case of static aqueous phase and HS/SPME, only numerical solutions were obtained.[3,4] There was no analytical expression relating the amount of the analyte sorbed by the fiber to its initial concentration in the sample. Hence, Ai proposed an SPME model for a two-phase system consisting of a sample solution and an SPME fiber.[5] Solving the problem of a more complex three-phase HS/SPME system with two interfaces (sample solution/gas phase and gas phase/fiber) is more complex. Two models have therefore been proposed. The first one is based on steady state kinetics assuming that the mass transfer rates at the two interfaces are the same.[6] Since analyte transfer rates across the interfaces may not be the same in real systems, Ai proposed an improved model for non-steady state mass transfer.[7] Providing a better description of the experimental data, the latest theoretical approach assumed that the analyte concentration in the headspace varies with the extraction time. The time variation of the analyte concentration in the headspace results in different rates of analyte evaporation from the solution and its extraction by the SPME fiber.

In trying to clarify the complex HS/SPME process and prove the practical benefit of general agreement between theory and experiment, the kinetic aspect of the process was included and a theoretical approach based on the HS/SPME kinetics is presented in this work. Experimental results obtained for pesticide residues extraction from water samples using the HS/SPME method were interpreted in terms of the developed model.

THEORETICAL TREATMENT

An HS/SPME process involving analyte mass transfer in three phases across two interfaces can be presented by the Equation:

$$S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} H \underset{k_4}{\overset{k_3}{\rightleftharpoons}} F \tag{1}$$

where S, H and F are the analyte concentrations in the sample solution, headspace (gas phase) and SPME polymer film (fiber), respectively; k_1, k_2, k_3 and k_4 are the rate constants of the processes occurring during the HS/SPME, namely analyte evaporation, condensation, sorption and desorption, respectively.

The rates of analyte migration in this system are:

$$-\frac{\mathrm{d}S}{\mathrm{d}t} = S' = k_1 S - k_2 H \tag{2}$$

$$\frac{\mathrm{d}H}{\mathrm{d}t} = H' = k_1 S - (k_2 + k_3) H + k_4 F \tag{3}$$

$$\frac{\mathrm{d}F}{\mathrm{d}t} = F' = k_3 H - k_4 F \tag{4}$$

where t is extraction time.

If S_0 represents the initial concentration of analyte in the sample, and V_s, V_h and V_f are the volumes of the sample, headspace and fiber, respectively, then:

$$S_0V_s = SV_s + HV_h + FV_f \quad (5)$$

Differentiating Eq. (4) and substituting H' and F' from Eqs. (3) and (4), the resulting equation becomes:

$$F'' = k_3[k_1S - (k_2 + k_3)H + k_4F] - k_4[k_3H - k_4F] \quad (6)$$

S and H can be expressed in terms of F and F' using Eqs. (5) and (4) and, therefore, Eq. (6) can be expressed as:

$$F'' + p_1F' + q_1F = k_1k_3S_0 \quad (7)$$

with the coefficients p_1 and q_1 having the form:

$$p_1 = k_1\frac{V_h}{V_s} + k_2 + k_3 + k_4 \quad (8)$$

$$q_1 = k_1k_4\frac{V_h}{V_s} + k_1k_3\frac{V_f}{V_s} + k_2k_4 \quad (9)$$

Eq. (7) is a second-order non-homogeneous linear differential equation. Its general solution, with integration constants C_1 and C_2, is:

$$F = C_1e^{-at} + C_2e^{-bt} + \frac{k_1k_3S_0V_s}{k_1k_3V_f + k_1k_4V_h + k_2k_4V_s} \quad (10)$$

with

$$a = \frac{p_1 - \sqrt{p_1^2 - 4q_1}}{2} \quad (11)$$

and

$$b = \frac{p_1 + \sqrt{p_1^2 - 4q_1}}{2} \quad (12)$$

Applying the initial condition, $F|_{t=0} = 0$, and replacing C_1 and C_2 with new constants, α and β, ($\alpha = -C_1$, $\beta = -C_2$), one obtains:

$$\alpha + \beta = \frac{k_1k_3S_0V_s}{k_1k_3V_f + k_1k_4V_h + k_2k_4V_s} \quad (13)$$

When the extraction time goes to infinity, Eqs. (10) and (13) become:

$$F^\infty = \frac{k_1k_3S_0V_s}{k_1k_3V_f + k_1k_4V_h + k_2k_4V_s} = \alpha + \beta \quad (14)$$

According to Eq. (13), Eq. (10) can be rewritten as:

$$F = \alpha(1 - e^{-at}) + \beta(1 - e^{-bt}) \quad (15)$$

In the treatment presented above, the mass transfers at both interfaces were taken as the rate determining steps. In reality, the mass transfer at one of the interfaces may play the major role and becomes the rate determining step.

DIFFUSION IN THE SPME FIBER AS THE RATE DETERMINING STEP

When diffusion of an analyte from the fiber surface to its inner layers is a much slower process than its evaporation from the sample, this diffusion can be taken as the rate determining step. It can be assumed that analyte partition equilibrium always exists between the sample and its headspace and that the analyte concentration in the headspace remains constant. According to Eq. (2), the partition constant of an analyte between the sample solution and its headspace (K_1) can be expressed as:

$$K_1 = \frac{S^\infty}{H^\infty} = \frac{k_2}{k_1} \tag{16}$$

Using Eqs. (5) and (16), H can be expressed as a function of F. On substituting the resulting expression for H in Eq. (4), the following relationship is obtained:

$$F' + p_2 F = \frac{k_3 S_0 V_\mathrm{s}}{V_\mathrm{h} + V_\mathrm{s} \dfrac{k_2}{k_1}} \tag{17}$$

with

$$p_2 = k_4 + \frac{k_3 V_\mathrm{f}}{V_\mathrm{h} + V_\mathrm{s} \dfrac{k_2}{k_1}} \tag{18}$$

The solution of this non-homogeneous linear differential equation is:

$$F = e^{-p_2 t} \left[C + \frac{k_3 S_0 V_\mathrm{s}}{p_2 (V_\mathrm{h} + V_\mathrm{s} \dfrac{k_2}{k_1})} e^{p_2 t} \right] \tag{19}$$

where C is an integration constant.

Applying the initial condition, $F|_{t=0} = 0$, the following expression is obtained:

$$F = \frac{k_3 S_0 V_\mathrm{s}}{p_2 (V_\mathrm{h} + V_\mathrm{s} \dfrac{k_2}{k_1})} (1 - e^{-p_2 t}) \tag{20}$$

When extraction time goes to infinity, Eq. (20) becomes:

$$F^\infty = \frac{k_3 S_0 V_\mathrm{s}}{p_2 (V_\mathrm{h} + V_\mathrm{s} \dfrac{k_2}{k_1})} = \frac{k_1 k_3 S_0 V_\mathrm{s}}{k_1 k_3 V_\mathrm{f} + k_1 k_4 V_\mathrm{h} + k_2 k_4 V_\mathrm{s}} \tag{21}$$

Finally, Eq. (20) can be rewritten as:

$$F = \frac{k_1 k_3 S_0 V_s}{k_1 k_3 V_f + k_1 k_4 V_h + k_2 k_4 V_s}(1 - e^{-p_2 t}) \quad (22)$$

Eq. (22) can be applied to the HS/SPME of volatile or semi-volatile analytes when the sample is heated above ambient temperature.

EVAPORATION FROM THE SAMPLE AS THE RATE DETERMINING STEP

Most analytes have low volatility when the HS/SPME is performed at room temperature. If evaporation of the analyte is much slower than its diffusion in the fiber, partition equilibrium is rapidly attained at the gas/fiber interface, with the evaporation process being the rate determining step**.** According to Eq. (4), the following solution follows:

$$F^{\infty} = \frac{k_3}{k_4} H^{\infty} \quad (23)$$

Using Eqs. (5) and (23) and expressing S and F in terms of H, Eq. (3) becomes:

$$H' + p_3 H = k_1 S_0 \quad (24)$$

with the coefficient p_3 expressed as:

$$p_3 = \frac{k_1 V_h}{V_s} + \frac{k_1 k_3 V_f}{k_4 V_s} + k_2 \quad (25)$$

If the initial and boundary conditions are $H|_{t=0} = 0$ and $H|_{t=\infty} = H^{\infty}$, respectively, the following solution of Eq. (24) is obtained:

$$H = \frac{k_1 k_4 S_0 V_s}{k_1 k_3 V_f + k_1 k_4 V_h + k_2 k_4 V_s}(1 - e^{-p_3 t}) \quad (26)$$

Finally, according to Eq. (23), the analyte concentration in the fiber is given as:

$$F = \frac{k_1 k_3 S_0 V_s}{k_1 k_3 V_f + k_1 k_4 V_h + k_2 k_4 V_s}(1 - e^{-p_3 t}) \quad (27)$$

EXPERIMENTAL

Materials

The fiber used (Supelco) was a fused silica fiber coated with a 100 μm poly(dimethyl siloxane) (PDMS) film. Before use, the fiber was conditioned in a gas chromatograph injection port as recommended by the manufacturer. A magnetic stirrer (Roth RCT Basic, Germany) and 8×3 mm stirring bars were used to mix the samples during extraction. The extraction was performed in 4 cm^3 vials (Supelco).

Standards

Pesticide standards, HCB (I), tefluthrin (II), heptachlor (III), aldrin (IV), chlorpyrifos (V), fenthion (VI) and bifenthrin (VII), (Dr Ehrenstorfer, Germany) were of 96–99.5 % purity.

Stock standard solutions of 1 mg cm^{-3} of each pesticide were prepared in acetone (J. T. Baker, USA). Working standard mixed solutions were prepared by diluting the stock solution with aceto-

ne. Water standard solutions were used for all SPME measurements. Highly purified deionized water (Purelab Option-R7, Elga, UK) was used for diluting the acetone standard solutions.

Apparatus

A gas chromatograph/mass spectrometer (GC/MS) was used as the detection device (CP–3800/Saturn 2200, Varian, Australia). A 30 m × 0.25 mm × 0.25 μm, VF-5ms column (Varian) was used. The injection port (1079 Universal capillary injector) temperature was set at 270 °C. After operating in the splitless mode for 9 min. (desorption time), the injector was set to the split mode (1:60). The GC was programmed as follows: initial temperature 120 °C, then increased to 170 °C at 10 °C min^{-1} and held for 20 min, increased to 280 °C at 15 °C min^{-1} and held for 2 min, increased to 290 °C at 10 °C min^{-1} and held for 10 min. Helium was used as the carrier gas at a flow rate of 1.1 ml min^{-1}.

The ion trap mass spectrometer was operated in the electron impact/selected ion monitoring (EI/SIM) mode. The ion trap and transferline temperatures were set to 220 °C and 250 °C, respectively. One specific pesticide ion was selected for detection and quantification, while a second one was used for confirmation. The ions inspected were as follows: 284 (214) for HCB, 177 (141) for tefluthrin, 274 (272) for heptachlor, 66 (293) for aldrin, 314 (286) for chlorpyrifos, 278 (109) for fenthion and 181 (165) for bifenthrin.

Procedure (sample preparation and analysis)

In order to determine the optimum extraction temperature, a one-hour extraction procedure was performed in the temperature range from 23 to 90 °C with the standard aqueous solution at a concentration level of 15 ng cm^{-3} of each pesticide. A linearity test was performed in the concentration range from 0.05 to 40 ng cm^{-3}. To confirm the proposed theoretical models, an aqueous standard solution of 10 ng cm^{-3} was used.

The aqueous standard solutions were prepared with an acetone content not higher than 1 % v/v, so as not to affect the extraction procedure.[2,8-10] In all experiments, 4 cm^3 vials were filled with 2 cm^3 of the standard aqueous samples. Each sample was analyzed in triplicate.

RESULTS AND DISCUSSION

On comparing Eq. (15) developed in this work with the Ai equation obtained for non-steady-state mass transfer, it is evident that both equations have the same form with two exponential terms, clearly confirming the correctness of the approach applied. When the diffusion of the analyte in the fiber was considered as the rate determining step, Eq. (22) was obtained, referring to the HS/SPME at elevated temperatures. In the case of analyte evaporation from the sample as the rate determining step, Eq. (27) was the final solution describing the extraction process at ambient temperatures.

In order to determine the optimum extraction temperature for each of the studied pesticides, extraction–temperature profiles were obtained in a temperature range from 23 to 90 °C and presented in Fig. 1. Increasing the extraction temperature obviously enhanced the amount of analyte sorbed by the fiber, which may be explained by increasing values of k_1 and k_3. In correlation with rapidly increasing values of k_2 and k_4, the amount extracted decreased at temperatures exceeding 80 °C for most of the investigated pesticides. For most of the studied pesticides, the maximum amount extracted in a single multi-residue analysis was achieved within the 60–80 °C temperature range and 60 °C was identified as the general optimum extraction temperature.

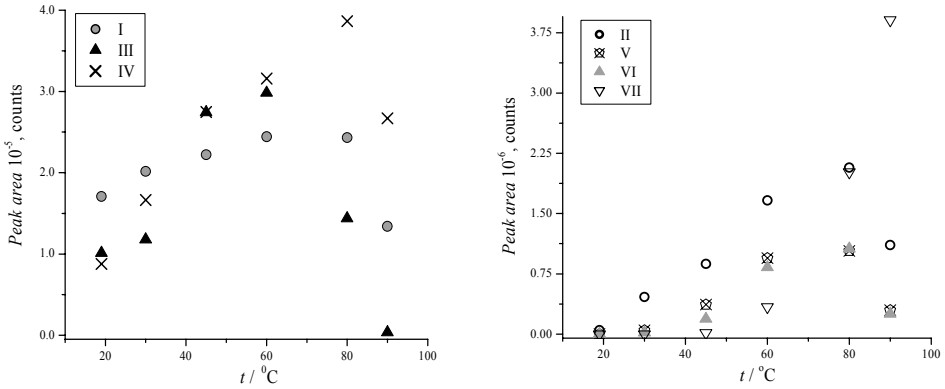

Fig. 1. HS/SPME–temperature profiles of the investigated pesticides (HCB (I), tefluthrin (II), heptachlor (III), aldrin (IV), chlorpyrifos (V), fenthion (VI) and bifenthrin (VII)); concentration: 15 ng cm^{-3}, extraction time: one hour.

The amounts of tefluthrin and aldrin extracted at 60 °C in relation to the extraction time are shown in Fig. 2. The obtained extraction time profiles had a shape well known in the literature and their dependences revealed a similar pattern for all the studied pesticides. Partition equilibrium was attained in periods up to 90 min. for all the studied pesticides, with the exception of bifenthrin.

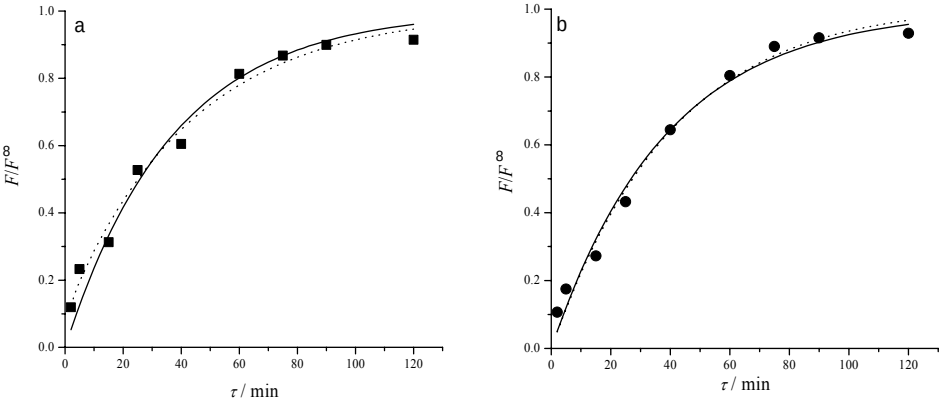

Fig. 2. HS/SPME–time profiles for a) tefluthrin and b) aldrin; extraction volume: 2 ml of aqueous standard solution, concentration: 10 ng cm^{-3}, mixing, temperature: 60 °C. The solid and dotted lines represent the fits of Eqs. (15) and (22), respectively.

Using a standard fitting procedure (OriginPro 6.1), the experimental data given in Fig. 2 were fitted to both Eqs. (15) and (22). Evidently, the experimental time profiles can be successfully interpreted using both theoretical equations. From the fitting procedures, and according to Eqs. (11) and (12), the parameters p_1, q_1 and p_2 were calculated and are listed in Table I. These parameters are dependent on the rate constants of the processes involved in the HS/SPME, and an increase

in temperature may be assumed to influence their increase. Evaporation of the analyte at the optimum extraction temperature becomes a very fast process and the model presented by Eq. (15) can be approximated with the simplified model given by Eq. (22).

TABLE I. List of parameters p and q derived from the experimental data fitted to Eqs. (15) and (22)

	Eq. (15)		Eq. (22)
Pesticide	$(p_1 \pm \Delta p_1)$ / min^{-1}	$(q_1 \pm \Delta q_1)$ / min^{-2}	$(p_2 \pm \Delta p_2)$ / min^{-1}
HCB	0.453 ± 0.027	0.029 ± 0.006	0.103 ± 0.022
Tefluthrin	0.962 ± 0.043	0.023 ± 0.002	0.030 ± 0.002
Heptachlor	0.596 ± 0.089	0.024 ± 0.003	0.032 ± 0.003
Aldrin	0.026 ± 0.003	0.026 ± 0.003	0.026 ± 0.001
Chlorpyrifos	0.024 ± 0.002	0.024 ± 0.002	0.019 ± 0.001
Fenthion	0.013 ± 0.001	0.013 ± 0.001	0.0056 ± 0.0002

It is obvious from Eq. (15) that the amount of extracted analyte can be expressed as a function of extraction time in the form of two exponential terms. According to Eq. (14), α and β should be proportional to S_0. Therefore, if t is held constant, F $\propto S_0$ in Eq. (15).

This relation is the key for quantitative analysis because it indicates that SPME quantification is possible before sorption equilibrium is attained. Also, having the same final form with a different parameter p included, the simplified models (Eqs. (22) and (27)) provide for quantification before absorption equilibrium is attained. Since partition equilibrium was attained within 90 min., practical application of the conclusion drawn was confirmed by relating the sorbed amounts to the initial analyte concentration in the sample over the 60-minute extraction time. Linear dependences, with regression coefficients ranging from 0.9895 to 0.9996, were obtained for pesticide concentrations in the ranges: 0.05–15 ng cm^{-3} (I), 0.05–25 ng cm^{-3} (II), 0.05–40 ng cm^{-3} (III), 0.05–40 ng cm^{-3} (IV), 0.05–40 ng cm^{-3} (V), 0.05–25 ng cm^{-3} (VI) and 0.05–15 ng cm^{-3} (VII). Relative standard deviation values for triplicate measurements were not higher than 19 %.

CONCLUSIONS

A kinetics-based theoretical treatment of the HS/SPME process was proposed. The same form of analytical expression as known from the literature was obtained. Simplified models, including analyte evaporation from the sample or analyte diffusion inside the fiber as the rate determining steps, were also presented. The HS/SPME experiments were performed with standard aqueous solutions of pesticides and the model developed successfully described the experimental data. Theoretical equations provide a linear relationship between the amount of analyte sorbed by the fiber and its initial concentration in the sample, enabling analyte quantification before sorption equilibrium is attained. The theoretical conclusion was confirmed experimentally by the linear dependences obtained for all the studied pesticides.

Acknowledgements: This work was part of the project No. TR-6890 B, supported by the Ministry of Science of the Republic of Serbia.

И З В О Д

МИКРОЕКСТРАКЦИЈА У ЧВРСТОЈ ФАЗИ («HEADSPACE» ТИП) У АНАЛИЗИ ОСТАТАКА ПЕСТИЦИДА – КИНЕТИКА И КВАНТИФИКАЦИЈА ПРЕ УСПОСТАВЉАЊА ПАРТИЦИОНЕ РАВНОТЕЖЕ

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Нови приступ теоријском разматрању процеса микроекстракције у чврстој фази (HS/SPME) је предложен и примењен у анализи остатака пестицида у воденим растворима. Модел се базира на трансферу масе кроз границе фаза, узорак/гасна фаза и гасна фаза/SPME полимер. Предложени модел даје директну пропорционалност између количине аналита апсорбоване на SPME влакну и његове почетне концентрације у узорку. Добијени израз указује да је квантификација могућа и пре достизања партиционе равнотеже. Модел је тестиран на екстракцији пестицида који припадају различитим класама органских једињења и добијено је очекивано слагање. Такође, линеарне зависности сорбоване количине пестицида од њихове почетне концентрације у раствору су добијене за време екстракције краће од оног потребног за достизање апсорпционе равнотеже.

(Примљено 9. фебруара 2006, ревидирано 12. марта 2007)

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